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NUTRITION REVIEWS

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NUTRITION REVIEWS

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CALCIUM REQUIREMENTS

The last revision of the recommended Daily Allowances of the Food and Nutrition Board in 1953 decreased the recommended allowance for calcium for adults from 1.0 g. to 0.8 g., and slightly increased the allowance for adolescents. These allowances still appear to be far out of line with the evidence available and with everyday experience.

The principal and simple fact is that no advantage has ever been demonstrated for the large intakes recommended, and no disadvantage has yet been demonstrated when much smaller amounts are habitually consumed. If the recommended allowances represented reasonable estimates of the actual need, there should be no lack of deficient material. Most of the world lives, from weaning onward through pregnancy and lactation, on diets which are not only very low in calcium but have the apparently bad calcium to phosphorus ratio of 1:10 (A. R. P. Walker, *Nutrition Reviews* **14,** *321 (1956)*). Still, the signs and symptoms of a dietary calcium deficiency syndrome in man remain undescribed.

Nearly all of the evidence upon which the allowances are based has been collected by the balance technic. There is now no reason to believe that these measurements bear any relation to calcium requirements of adults. They simply provided a round-about way of estimating what the past calcium intake of the individual has been (D. M. Hegsted, *Methods of Evaluation of Nutrition Adequacy and Status, p. 103, National Research Council (1954)*). In children one might expect, on theoretical grounds, that balance technics might have more significance. However, the degree of positive balance often reported is so high that it appears unreasonable (J. O. Holmes, *Nutr. Abstr. & Rev.* **14,** *598 (1944–45)*). We may suspect that some of these high retentions might be related to differences in the calcium intake before and during the study. Also, it does appear (D. M. Hegsted, *J. Am. Dietet. Assn.* **33,** *225 (1957)*) that balance studies often give positive retentions which are impossibly high and thus of doubtful significance as indications of requirement. As will be pointed out below, maximum retention cannot be equated necessarily with desirable retention. In any event, even in children and adolescents where calcium requirements must be higher than in the adult, no evidence of calcium deficiency has been reported regardless of the calcium intake.

The highest calcium requirements would appear to be during lactation, since a liter of human milk contains about 300 mg. per liter. Osteomalacia has been reported in pregnant and lactating women in some countries. This disease apparently responds to vitamin D therapy but not to calcium therapy (R. R. Hannon *et al., Chinese Med. J.* **48,** *623 (1934)*; *Ibid.* **49,** *1 (1935)*). Hence, it appears to be due to a vitamin D deficiency, perhaps partially induced by a relatively high vitamin D requirement when the calcium intakes are low and the calcium requirements are high. Most women seem to get enough exposure to sunlight. It is clear that the majority of women throughout the world must be in strong negative balance during lactation. This must mean that women, like dairy cattle, can draw upon their calcium stores during lactation and replenish them afterward. There is no reason to consider this bad or unphysiological. In dairy cattle where the losses are much larger, calcium supplements are not required (H. T. Converse, *U.S.D.A. Bull. No. 1092 (1954)*).

The long-term studies of Sherman and his associates, which showed that increased intakes of calcium improved reproductive performance and lengthened the life span of

rats (*Nutrition Reviews* **10,** *97* (*1952*); **11,** *177* (*1953*); **15,** *84* (*1957*)), have greatly influenced the thinking of nutritionists in this field. However, as has recently been pointed out, (*Nutrition Reviews* **15,** *84* (*1957*)) under some conditions 0.4 or 0.6 per cent of calcium were equally adequate while under other conditions intakes above 0.5 per cent Ca were detrimental. An optimum intake thus appears to be neither too much nor too little. At present we have no way to translate these studies quantitatively into human experience. The growth requirements of the rat for calcium are tremendous compared to those of man. A diet containing 0.5 per cent Ca would supply 2.5 g. per 2000 calories. Would one gram per man per day correspond to 0.5 per cent for the rat, or is it relatively higher or lower?

The experiments of Henry and Kon (*Brit. J. Nutrition* **7,** *147* (*1953*)) deserve mention. These studies showed quite convincingly that rats adapted to low calcium intakes were more resistant to calcium depletion when they became old adults. If decalcifications, which occur in old age, have any relation to calcium intake (which is doubtful), adaption to low intakes may actually be desirable. Similar suggestions come from another area. J. M. Boda and H. H. Cole (*J. Dairy Sci.* **37,** *360* (*1954*); **39,** *66* (*1956*)) showed that cattle were protected from milk fever by feeding them a *low* calcium diet prior to parturition. Presumably by lower levels of calcium intake the parathyroids were stimulated and kept active. Thus, when the need for calcium was great, the mechanisms for mobilizing it were available. The philosophy that all "nutritional stress" is bad, or that the body should be kept loaded in case deficient periods come along, deserves a much closer look. It may be completely wrong.

Finally, a number of papers have appeared on metastatic calcification associated with high calcium and alkali intakes in ulcer patients (*Nutrition Reviews* **13,** *199* (*1955*)). While the alkali intake is probably of more importance than the calcium intake, the occurrence of this syndrome does serve to emphasize that excesses may be as disadvantageous as deficiencies. High intakes of vitamin D are common in children (*Nutrition Reviews* **15,** *74* (*1957*)) and hypercalcemias are not uncommon in some parts of the world (*Nutrition Reviews* **14,** *338* (*1957*)). Although the role or relative importance of the calcium intake in these hypercalcemias is still unknown, it is logical to assume that it plays a part. Low calcium diets are used therapeutically.

The role of nutrition is to assure, insofar as possible, that all people receive an optimum diet whether this means increasing the intake of certain nutrients or decreasing the intake of others. Either approach is equally the business of those interested in nutrition. The trends in nutrition during the past decade clearly suggest the need for more work and consideration of the upper limits of adequacy. With regard to the nutrient calcium, there seems to be no cogent reason for attempting to raise intakes that appear low by current standards, nor for lowering intakes such as are common in the United States. Until such data are available, dietary recommendations should make this clear.

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WATER AND LIFE IN THE DESERT

Everyone recognizes that water is the most important nutrient for survival (*Nutrition Reviews* **8**, *1* (*1950*)). The importance of water is most apparent in desert areas where it is chronically in short supply.

The desert and semi-arid areas of the

world frequently are extremely warm and dry during long periods of the year. The combination of short water supplies and high rates of water loss would favor the development of dehydration.

Studies on the water requirement of men in desert areas was stimulated by the necessity of training troops during World War II for combat in North Africa. These studies by E. F. Adolph and associates (*Physiology of Man in the Desert. Interscience Publishers, Inc., N. Y. (1947)*) showed that it is impossible to train or adapt a man to work in the desert on a restricted water ration. The University of Rochester group and others observed that dehydration under such circumstances is associated with fatigue, a variety of minor psychological alterations (irritability, nausea, lassitude, etc.) and a number of physiological changes (such as increased heart rate and rectal temperature, and loss of body weight).

Since there are marked individual variations in caloric expenditure, it is obvious that the amount of water required to maintain normal hydration will vary in different individuals, and in the same individual from day to day. The actual amount of water required to maintain normal hydration can be ascertained best by noting the amount of water required to maintain a normal or nearly normal urine volume. Excess water was shown by Adolph and associates (*Loc. cit.*) to have no, or at most only a very slight, influence on the volume of sweat. The excess promptly appeared as an increased urine volume and a reduced specific gravity. It was observed by the above workers that most of their subjects (soldiers in armored divisions) consumed most of their water with their meals, and the only feasible means of economizing on the water requirement was to reduce the need for sweating. They reported that a man in the North African desert requires approximately three liters of water per day as a minimum. Strenuous physical activity may increase the requirement to seven liters, with an average between four and five liters.

The studies reported by Adolph and associates are important and valuable contributions to our knowledge of water requirements, but they have been made with subjects who, relatively speaking, were newcomers to the desert. For this reason, the preliminary studies reported by T. W. Burns (*J. Applied Physiol.* **9,** *287 (1956)*) are of considerable significance. Burns studied the tribesmen in the southern part of the Eastern Desert in Egypt. The study was made in August (1954) which is the hottest period of the year in an area that is considered one of the hottest in the world. This area, bordering on the Red Sea, has an estimated annual rainfall of 8 to 10 mm.

In spite of the forbidding circumstances, an Arab tribe, the 'Ababda, lives there. The procurement of water is the primary necessity of these wandering people. Not only are wells far apart, but the water varies from being moderately brackish to being so bitter and saline as to be fit only for cattle. When even the limited potable water fails, the native tribesmen are known to drink the brackish water.

Water samples from eight wells in the above desert area showed total electrolyte concentrations ranging from 33 to 146 mEq per liter. The sodium content ranged from 14 to 58 mEq per liter, and the chloride from 20 to 122. (The latter is equivalent to a concentration of 0.7 per cent sodium chloride.)

Burns (*Loc. cit.*) points out that F. W. Shotton (*Water and Water Eng.* **49,** *28 (1946)*) indicated that the maximum salinity for potable water in Egypt, according to the British, was 0.35 per cent sodium chloride. Burns' report suggests that water of higher salinity is being used by the 'Ababda tribespeople for fairly long periods of time. In spite of the high salinity, urine specimens collected from the native tribesmen had specific gravities below 1.03, which was actually lower than the specific gravities of

the urines secured from members of the research team who used water liberally throughout the day. The concentration of sodium chloride in the urine was essentially the same in the tribesmen and the research workers. This would indicate that the tribesmen who travelled the caravan routes in that desert area were able to secure enough water for their needs.

The concentration of nitrogen and potassium was considerably greater in the urine of the research workers than in that of the tribesmen. The latter difference was probably a reflection of dietary intakes, since the native tribesmen were on a low protein diet. Their diet was reported to be largely carbohydrate, made up of bread prepared from barley flour. By this means, the natives were able to reduce urinary nitrogen excretion and thus avoided burdening the kidney with a high solute load.

The studies indicated that the native tribesmen were well adapted to the conditions of the desert. They reduced to a minimum their need to dissipate heat by controlling their expenditure of energy.

These studies are important and significant in focusing attention upon a problem that has been too frequently neglected, especially from the nutritional and physiological standpoints. There are large areas of the world where water is at a minimum, and yet there are peoples who live in these areas without manifesting any overt physiological symptoms of water deficiency. A quantitative study of the water intake of these natives and their urinary excretion would provide data on which one could decide whether there was any "long-term" adaptation to a restricted water supply.

A medical problem that has been associated with some of the areas where water supplies are short is a high incidence of kidney stones. L. W. Pierce and B. Bloom (*J. Urology* **54,** *466 (1945)*) reported a high incidence of kidney stones among American troops who were stationed in "one of the larger desert areas of the world," where the "summers are long and severe with exceptionally high temperatures." The incidence of kidney stones in this area increased during the hottest months. On the plains where the temperatures were higher than in the mountains, the incidence was higher and it decreased in the cooler months. The urine flow was reported to be low during the summer. Some men abstained from voiding thirty hours with no apparent discomfort. These American soldiers were on standard army overseas rations. The incidence of kidney stones was ten times more common among white than among Negro troops. The stones were either calcium oxalate or calcium phosphate, with some being mixtures of the two.

What little epidemiological work has been done suggests that there is a high incidence of kidney stones among inhabitants of those parts of the world where water is in inadequate supply (C. C. Higgins, *Urolithiosis, p. 767 in Urology, M. Campbell, ed. W. B. Saunders Co. Philadelphia (1954)*). The assumption is frequently made that the absence of adequate amounts of water is primarily responsible for the kidney stone formation. The low water intake supposedly results in a urine of high specific gravity which then favors the precipitation of the more insoluble salts that are present in sufficient amounts to form stones. The work of Burns (*Loc. cit.*) casts suspicion on such an assumption, since the specific gravity of the urine collected from the native tribesmen did not suggest any unusual concentration of the urine. Unfortunately, Burns' work was acknowledged by him as being nothing more than a preliminary report, and he hoped that further studies of the same subject would be made by subsequent investigators. It can only be hoped that the initiation of such work will not be too long delayed.

IRON DEFICIENCY ANEMIA AFTER PARTIAL GASTRECTOMY

The occurrence of anemia after partial gastrectomy has been variously estimated at from 10 per cent to 50 per cent. These anemias are of the iron-deficiency type, with typical hypochromic anemia and low serum iron concentration. The cause of this anemia has been attributed to bleeding, to the postoperative achlorhydria and consequent poor iron absorption, and to invasion of the upper intestine by bacteria. P. A. Owren (*Acta. Clin. Scandinav.* **104,** *207 (1952)*) demonstrated that iron treatment orally was completely effective in correcting the anemia in two patients. In both patients, tablets of ferrous chloride were ineffective when the patient was ambulatory but led to the expected rise in hemoglobin when the patients remained at bed rest. This suggested that rapid gastric evacuation prevented the absorption of the tablets. Therapy with iron in a pulverized form was effective in the ambulatory patient, demonstrating that under these conditions iron absorption was satisfactory.

M. D. Smith and B. Mallett (*Clin. Sci.* **16,** *23 (1957)*) compared the absorption of iron before and after partial gastrectomy in 22 patients, and also studied 27 other patients who had previously had a partial gastrectomy performed. They gave 5 mg. of ferrous sulfate labeled with 5 microcuries of Fe^{59} in a solution containing 50 mg. of ascorbic acid to maintain the iron in the ferrous state. Fecal radioactivity was followed until less than one per cent of the administered dose was present in one day's collection. Red cell radioactive iron also was determined until a constant level was reached.

Iron absorption ranged from 8 to 42 per cent (mean 27) in a group of control patients. The patients with peptic ulcer before surgery had essentially the same absorption of iron. When these subjects were tested six weeks and six months after partial gastrectomy, absorption was unchanged.

The authors also examined the iron absorption in 19 patients who had a partial gastrectomy one to five years previously and were not anemic. Iron absorption was normal in this group. Eight additional patients with an iron deficiency anemia were shown to have increased absorption of iron.

These tests constitute good evidence that the iron deficiency anemia after partial gastrectomy is not due to poor absorption. Although absorption of iron from a food mixture is possibly less than under the ideal conditions of this study, the total amount of iron used was small and comparable to that present in a single meal. Further studies of iron absorption are needed using food iron rather than ferrous sulfate.

The reasons for the anemia are still not defined. Blood loss cannot be demonstrated in many of these patients. The rapid gastric emptying and short transit time as noted above may tend to decrease absorption. The known decreasing gradient of iron absorption in the distal small bowel would favor such an interpretation. However, apart from the mechanisms concerned, the problems of iron deficiency anemia again emphasize the many metabolic derangements which may occur in the absence of normal gastric function.

THE D-XYLOSE ABSORPTION TEST

The diagnosis of the malabsorption syndromes has prompted the devising of many tests of small intestinal absorption. It was suggested by O. M. Helmer and P. J. Fouts

(*J. Clin. Invest.* **16,** *43* (*1937*)) that D-xylose might be useful in this regard. They then demonstrated defective absorption of this sugar in three cases of non-tropical sprue.

There are sound theoretical reasons for using this pentose as a test agent. It is not absorbed against a concentration gradient as is glucose, and is not normally present in blood. As it is non-fermentable, its determination in the presence of glucose is easy. It is unaltered by the liver and a constant proportion is excreted in the urine. The rate of urinary excretion is proportional to the plasma concentration and independent of urine flow.

F. S. Brien, *et al.* (*Gastroenterology* **20,** *287* (*1952*)) studied the blood levels and urinary excretion of D-xylose after ingestion of 25 g. of the sugar. When the D-xylose in the blood was determined at hourly intervals, the D-xylose tolerance curves were quite reproducible among patients. The coefficients of variation of the hourly blood pentoses were considerably less than those for comparable glucose or fructose tolerance tests. The urine excretion also was quite constant and 6.14 ± .70 g. were excreted in five hours. The authors concluded that the constancy of the changes in blood xylose concentration and urinary xylose excretion made this sugar a good test substance for absorption.

L. P. R. Fourman (*Clin. Sci.* **6,** *289* (*1948*)) used this test in three patients with idiopathic steatorrhea and three with tropical sprue. These patients excreted only 2.0 g. in five hours; his normal control patients excreted 6.1 g. in the same length of time. He, too, felt that this was a valuable diagnostic test in the malabsorption syndromes.

More extensive studies have been made by J. A. Benson, Jr., *et al.* (*New Engl. J. Med.* **256,** *335* (*1957*)) who also administered 25 g. of D-xylose to their patients, and followed both urinary xylose excretion and blood xylose levels. The five-hour urinary xylose excretion was 6.5 g. in 24 normal subjects and 1.3 g. in 28 patients with non-tropical sprue. With only four exceptions there was no overlap between the two groups. When a therapeutic effect was achieved in the patients with sprue by either a gluten-free diet or cortisone, the urinary xylose excretion increased to 3.0 g. in five hours. Five patients with deficiency of pancreatic enzymes displayed normal absorption of xylose.

The authors also showed that this test is not specific for the malabsorption syndromes as it was also abnormal in a miscellaneous group of patients with regional ileitis, multiple intestinal resections, and multiple diverticula. Thus, the available absorbing surface of the intestine is also reflected in the xylose absorption test.

In contrast to the results of Brien, *et al.* the blood xylose curves in the patients with malabsorption syndromes were found to overlap the normal patients. It was therefore a less valuable diagnostic maneuver than the urinary excretion.

All of the investigations have supported the view that the D-xylose absorption test offers many advantages over other tests for the malabsorption syndromes. It is simple, reproducible, and no venipunctures are required. The test should receive wider application.

VITAMIN D IN THE NEWBORN

The mechanism of action of vitamin D has been studied in the adult by many investigators. H. E. Harrison and H. C. Harrison (*J. Clin. Invest.* **29,** *47* (*1941*)) showed that vitamin D acts on the renal tubules enabling animals to resorb the phosphate filtered by the glomeruli. Absence of the parathyroids results in maximum absorption

of phosphate by the renal tubules. When parathyroid extract is administered, the absorption is much reduced, with a resultant phosphorus diuresis. Thus, vitamin D appears to have an effect antagonistic to that of parathormone. However, it was found later that vitamin D in excessively large quantities produces parathormone-like results. Thus, vitamin D apparently has a dual effect, depending on the dose given.

Some years ago, J. B. Pincus and I. F. Gittleman (*Am. J. Dis. Child.* **51,** *816 (1936)*) carried out studies on an infant having neonatal tetany. The child was on breast milk for the first five weeks postpartem and was then weaned and placed on a whole-milk mixture. Antirachitic supplement had been given since the fifth week. During the sixth week the infant developed convulsions. Serum calcium was 6.2 mg. per cent and serum phosphate was 8 mg. per cent. When the intake and output of calcium and phosphorus were measured in two preliminary periods of three days each, it was found that the partition of calcium and phosphorus in the urine and the feces resembled that seen in cases of hypoparathyroidism. When parathyroid extract was administered, a marked phosphorus diuresis occurred and the serum calcium and phosphorus became normal. When parathyroid extract was withheld, the convulsions recurred, and the serum calcium and phosphorus returned to the previous abnormal levels with abnormal partition of calcium and phosphorus in the urine and feces. The tentative conclusion made was that tetany in the newborn in such cases resembled adult states of hypothyroidism.

H. Bakwin (*Am. J. Dis. Child.* **54,** *1211 (1937)*) subsequently found that administration of inorganic phosphorus to newborn infants in the first week following birth resulted in elevation of the serum phosphorus level to such a degree that the calcium was affected reciprocally. It has recently been established by H. L. Barnett (*Pediatrics* **5,** *171 (1950)*) and others that the infant kidney glomerular filtration rate is only 33 to 40 per cent of that found in the normal adult. This means that the infant will be more prone to retain phosphorus and thus, conceivably, more likely to have a subnormal serum calcium. It has further been shown that breast milk, which contains a calcium to phosphorus ratio of 2 as well as relatively low concentrations of those ions, protects newborn infants against the occurrence of neo-natal tetany.

In a more recent study of the effects of various diets on the calcium and phosphorus levels of infants during the first week following birth, J. B. Pincus, I. F. Gittleman and A. E. Sobel (*N. Y. Acad. Sci.* **64,** *279 (1956)*) found that when cow's milk low in vitamin D was given to infants, a lower incidence of hypocalcemia was observed than in infants fed cow's milk enriched with the vitamin.

Infants in the first weeks after birth were also placed on a processed milk formula containing no vitamin D. These infants were compared to others receiving similar diets enriched with 400 units of vitamin D per quart and also with a group of infants receiving the powdered milk mixture supplemented with 600 units of vitamin D daily. The incidence of hypocalcemia was significantly higher in the group receiving vitamin D. A group of breast-fed infants given a supplement of 600 units of vitamin D also had a tendency toward higher phosphorus and lower calcium but not to clinical hypocalcemic levels.

It thus appears that, when given to infants during the first week post partem, vitamin D is not beneficial to the infant from the standpoint of serum calcium and phosphorus levels, since the tendency to hypocalcemia is increased.

The authors conclude further that if the assumption is made that vitamin D acts on the renal tubules as an antagonist to parathormone, an explanation can be made for the paradoxical effect of vitamin D in the newborn. A state of hypoparathyroid-

ism of varying degrees of severity possibly exists during the first week or so after birth. Administration of vitamin D in the accepted therapeutic doses during this interval would tend to aggravate such a hypoparathyroid state.

THE PAROTID GLANDS AND NUTRITIONAL DISEASES

Although changes in the parotid glands associated with vitamin A deficiency are well established, enlargement in other nutritional diseases has not often received attention. Because of the similarity of the parotid glands to other digestive glands (*e.g.* the pancreas) one may wonder whether worthwhile observations have been overlooked because of the infrequent autopsy or biopsy study of these organs.

E. N. Rothbell and J. J. Duggan have recently drawn attention to parotid enlargement in liver disease (*Am. J. Med.* **22,** *367* (*1957*)). They report six clinical cases; four were alcoholic patients with inadequate diets and the other two were believed to be so. Three males and three females, their ages ranged from 32 to 52 years. Five had liver biopsies; two showed fatty livers, three showed portal cirrhosis, and a sixth had good clinical evidence of this latter disease. Biopsies of the parotid glands were obtained from four of the patients; all showed "fatty infiltration" (adipose tissue between groups of acini) with apparently normal glandular and duct tissue. All six patients were obese, raising the question of whether the parotid enlargement was a manifestation of obesity. The authors discounted this possibility, probably too lightly. Three patients had a diastolic arterial hypertension and three (two of the hypertensive patients and one other) had impaired glucose tolerance. Because parotid enlargement "in Europe and America. . . . has appeared in clinical settings of liver disease, obesity, diabetes mellitus . . .", the authors conclude that its significance seems to be much the same as that of fatty liver, "pointing to disturbed nutrition or metabolism but not defining the disturbance".

A similar report by S. J. Wolfe, W. H. J. Summerskill and C. S. Davidson (*New Engl. J. Med.* **256,** *491* (*1957*)) appeared at about the same time as that of Rothbell and Duggan. Wolfe and his colleagues describe sixteen patients admitted to the Boston City Hospital with unequivocal evidence of portal cirrhosis and bilateral asymptomatic parotid swelling. Two individuals also had swelling of the submaxillary glands. Nine patients died of liver failure with severe jaundice. Another died of other causes. Parotid swelling diminished in three survivors as their clinical state improved. Two patients had increased serum amylase values. One of these had a clinical picture suggesting acute pancreatitis, but autopsy showed only dilatation of the pancreatic ducts. No mention is made of the parotid histology; one infers that the glands were not sectioned. The authors conclude that parotid enlargement is indeed frequent in chronic alcoholic individuals, most of whom have hepatic cirrhosis, and that "the change in these subjects may be related to those reported in malnourished persons".

The relation between malnutrition and the parotid gland has been reviewed in this journal (*Nutrition Reviews* **13,** *302* (*1955*)) and the conclusion offered that "a high incidence of the type of parotid enlargement described . . . would seem to be yet another yardstick for severity of malnutrition".

The literature on chronic or "habitual" parotid enlargement is a heterogeneous one and its study made difficult by the sparsity of histologic observations. If one excludes inflammatory, neoplastic, infiltrative, obstructive, developmental and other known causes of parotid enlargement, there remains a residue which comprises at least three

types of enlargement: adipose tissue replacement, hypertrophy, and hyperplasia. Individual reports on such cases, usually bilateral, often of years' duration and commonly not involving the other salivary glands, are documented in the older literature and summarized (for example, in F. J. Lang's monograph in Henke and Lubarsch's Handbuch der speziellen pathologischen Anatomie und Histologie (Vol. 5/2, pp. 1–210. Berlin, Springer, 1929)). Adipose tissue replacement associated with parotid atrophy is found in obesity, in aged individuals, and in patients who have died with chronic debilitating diseases. The process thus appears to be the replacement of an atrophying parenchyma, much as in pancreatic atrophy or aplastic bone marrow. A variety of random associated diseases has been reported, usually isolated cases, without evident common etiology. Lang reports a 56-year-old male with atrophic cirrhosis and several years' swelling of the parotid glands which proved histologically to have pure hypertrophy (without fatty tissue). Cases of true hypertrophy have been reported in obesity, and parotid enlargement has been reported in diabetes and more than occasionally in alcoholics.

The careful paper of M. R. Kenawy, of Cairo (*Tr. Roy. Soc. Trop. Med. Hyg.* **31,** *339* (*1937*)) deserves attention. The author described epidemic, chronic, painless enlargement of the parotids as common in Egypt, affecting "2% of the poorer agricultural workers" in some districts. He was careful to exclude obstructive and inflammatory enlargements from the series. Of the 100 cases (among hospital inpatients) he reported, 65 had, in addition, pellagra, ten had manifest or latent diabetes, and six had cirrhosis of the liver. The submaxillary glands were bilaterally enlarged in eight cases and the lachrymal glands were so in one case. No derangement of gastric or pancreatic function was observed. There was no diminution in ptyalin activity in the 29 cases studied. After pilocarpine stimulation, parotid secretion was much increased compared to the normal, and the increase was in proportion to the degree of parotid enlargement. Very few patients complained of sialorrhea. Sialograms were normal in half of the cases. Fusiform and segmental dilatation of the medium and larger ducts were seen in 8% of the cases and are well shown in the article. Other cases had spherical dilatations of the ends of small ducts. Microscopic examination of three autopsy and two biopsy specimens showed "chronic hyperplasia with entire absence of any inflammatory changes", giving the appearance of an actively functioning gland. The photomicrographs show closely crowded acini, dilated ducts, and no adipose tissue. Kenawy concluded that "hyperplasia occurs in response to some increased functional demand on the glands . . . from some need of the body secondary to some deficiency".

Scattered through the literature are a number of reports of parotid enlargement, in addition, in malnourished non-pellagrins in Madagascar and Algeria, a number of other areas of the world, and in concentration camps and institutions.

Although various authors have reported stigmata of different nutritional deficiencies in association with parotid enlargement, a single deficiency has not been incriminated, and it seems more likely that a heterogeneous group of nutritional inadequacies, caloric, protein, and vitamin, has been culled. The association of diabetes mellitus with painless parotid enlargement has been noted in a number of reports, as has obesity.

An altogether excellent clinico-pathologic correlation and discussion of "the large parotids of the alcoholic cirrhoses" is provided by H. Bonnin, G. Moretti and A. Geyer (*Presse Med.* **62,** *1449* (*1954*)). These authors wisely excluded obese subjects from study. Their series consisted of 173 patients with parotid explorations, some with confirmed cirrhosis, others with "alcoholic precirrhosis (steatosis)", and some with needle biopsy of the liver. The authors give a good

clinical description of the parotids which, to their surprise, they found enlarged in 80% of their series. They found amylase activity of parotid secretion increased, in distinction to the striking fall in serum amylase and pancreatic enzymes well recognized in malnutrition syndromes and tropical cirrhoses. Bonnin and his co-authors characterized the histologic features (well shown in their photomicrographs) as follows: (1) The "aquipare" appearance; a rather characteristic exhaustion of zymogen granules with the cells filled with clear "aqueous vacuoles". The authors did not find this in other morbid states. (2) "Caricatural overactivity;" an exaggeration of the secretory cycle seen in alternating functional activity of sharply differing neighboring areas of the glands: a) a zymogen stage, with parotid cells packed with large granules, b) a serous stage with engorgement, and c) the "aquipare" stage. In addition, localized or diffuse hyperplasia of the glandular epithelium was noted. Interstitial adipose tissue was most frequently described as normal in amount, though occasionally increased in obese cirrhotics. Fibrosis was generally minimal. The authors conclude that "the increase in volume of the parotids in cirrhosis appears as an adaptation of the glandular tissue to an increase of salivary function". The characterization of an exhaustion stage and "the exaggeration of the visible phases of secretion in the preliminary granular and serous stages, confer on the parotids of the cirrhotic a remarkable specificity". They believe "denutrition" is probably responsible for the parotid changes. This would explain the familial incidence, the association with beri-beri and pellagra, and the frequency among native races in hot countries where manioc diets, or others lacking in protein, are often associated with cirrhosis.

Although parotid enlargement is not part of our concept of kwashiorkor, J. N. P. Davies points out (*Lancet* **I**, *317* (*1948*)) that in treated cases "almost the first sign of clinical improvement is a swelling of the parotid and other salivary glands at first thought to be mumps".

One may speculate that the sequence in nutritional parotid enlargement is hypertrophy (with excess secretion of saliva and amylase), exhaustion, atrophy, and adipose tissue replacement. The last is independently increased by obesity. The incompletely atrophied parotid, with suitable restoration of a nutritious diet, would be capable of responding to physiologic stimuli by increased secretion and hypertrophy, which is clinically visible in treated kwashiorkor. Clinical evidence suggests parallel changes in the pancreas (which shows considerable fibrosis in distinction to the parotid glands). If kwashiorkor and Laennec's cirrhosis are similarly caused, we may agree with Bonnin and look to protein undernutrition as a major factor in the parotid enlargement so little noted in the American literature.

It is quite possible that, in a given patient, cytologic changes might have occurred and disappeared long before biopsy, as the gross lesion may have developed over a period of years. This might account for the shrinkage of acini and preponderance of adipose tissue in the parotids of obese alcoholics. Acute changes would be more readily demonstrable in controlled animal experiments. Meanwhile, one hopes that future work on parotid enlargement in nutritional disease will continue to be documented with biopsies. The lack of histologic parotid changes reported in cystic fibrosis of the pancreas and in kwashiorkor should not deter search for them in these and other diseases associated with nutritional abnormalities, especially of the pancreas.

In summary, painless bilateral parotid enlargement has been reported in association with alcoholism and liver cirrhosis. The association with other nutritional deficiencies is reviewed; a nutritional component at fault is not identified, but protein is suspect.

CYSTINURIA

Cystinuria was first recognized in 1810 by Wollaston who noted the unusual composition of a bladder stone. In 1923, Garrod characterized cystinuria as one of the hereditary errors of metabolism and suggested that the cystinuric lacked an enzyme necessary for the first step in the metabolism of cystine, thus permitting excess cystine to build up and subsequently be excreted in the urine. It was soon apparent, however, that this simple explanation was not correct, but detailed elaboration of the pathogenesis of the disease had to await the development of more precise analytical techniques.

C. E. Dent and G. A. Rose (*Quart. J. Med.* **20,** *205* (*1951*)) utilized paper chromatography for the study of amino acid metabolism in cystinurics. Fifteen patients with cystinuria demonstrated nearly constant urinary excretion of large amounts of lysine and arginine as well as cystine. The amino-aciduria was not generalized, however, and did not overlap other diseases with amino-aciduria, such as Wilson's disease and Fanconi syndrome. As the plasma level of cystine is normal in cystinurics, attention was focused on the renal tubule as the locus of the biochemical alterations resulting in the disease (*see Nutrition Reviews* **13,** *198* (*1955*)). The occurrence in the urine of such different amino acids as cystine, lysine and arginine would not likely be due to a single error of metabolism similarly affecting each of these compounds. However, certain gross structural similarities of the three amino acids, such as the presence of two amino groups separated by a chain of four to six atoms may implicate a common tubular mechanism.

In an extension of this study, Harris *et al.* (*Ann. Human Genetics* **19,** *196* (*1954*)) examined the urines from 28 cystinuric patients and 121 of their relatives for cystine, lysine, arginine and ornithine. This last amino acid was recognized by ionophoresis only. In the urines of all of these subjects there was a close correlation between the cystine and lysine contents over the whole range. The arginine excretion with respect to either cystine or lysine gave an entirely different pattern. The arginine output remained near normal until the urinary cystine output reached 250 mg. per gram of creatinine or the lysine excretion reached about 500 mg. per gram of creatinine. Above these apparent threshold values, large increases in arginine output were found. It was also noted that when arginine excretion was high, ornithine was present. These findings again suggest a common tubular resorptive mechanism for the four amino acids. When the process fails completely, all four amino acids appear in the urine in large amounts. When it is only partially deranged, ornithine and arginine are preferentially reabsorbed.

E. B. Robson and G. A. Rose (*Clin. Sci.* **16,** *75* (*1957*)) undertook to provide direct experimental evidence in support of a common renal tubular pathway for the reabsorption of these amino acids. They attempted to overload the tubular reabsorptive capacity for lysine by infusing lysine intravenously and studying the effect upon the reabsorption of cystine, arginine and ornithine. This study was performed in normal patients, stone-free cystinuric patients, a semi-cystinuric patient (*v.i.*) who excreted smaller amounts of cystine and lysine only, a patient with adult Fanconi syndrome and heterozygous carriers of the fully recessive forms of cystinuria and Fanconi syndrome. Plasma ultrafiltrates and urines were examined for amino acids by paper chromatography and electrophoresis. Arginine and lysine were estimated by microbiological assay and cystine polarographically.

None of the patients reported any ill effects from the five-gram infusions of lysine. There were only minor changes in the plasma levels of cystine, arginine and ornithine so that the increased urinary excretion

of these amino acids was explicable only on the basis of altered renal tubular activity. The normal patients demonstrated gross increases in the urinary excretion of cystine, lysine, arginine and ornithine, thus supporting the hypothesis of a common tubular mechanism.

Patients with cystinuria and the patient with Fanconi syndrome displayed only minor changes, consistent with the view that the administered lysine could not further depress the deficient reabsorption of the other amino acids. The unaffected heterozygous carriers of cystinuria and Fanconi syndrome demonstrated the same changes as the normal patients.

As there were small increases in the excretion of other amino acids in all patients, equimolecular quantities of glycine were infused into a normal person. There was no change in the excretion of the four amino acids affected in cystinuria.

This series of papers greatly clarifies our understanding of cystinuria. The availability of these methods also offered an excellent opportunity to investigate the genetics of the disease. H. Harris and L. Warren (*Ann. Eugenics* **18,** *125 (1953)*) made an extensive analysis of the genetics of cystinuria in 232 individuals belonging to 21 families. These families were selected by the presence of at least one member of the family with cystine stone formation. Because of the difficulty in obtaining accurately-timed 24-hour urine collections, cystine-creatinine ratios were used in the analyses.

The data revealed two distinct types of families. In one type there were two separate classes, normals and cystinurics. There was no overlap in the values for cystine excretion between these two classes. Among the cystinuric members, some were stone-free. The inheritance of cystinuria in these families could be readily interpreted by the hypothesis that the cystinurics were homozygous for a rare recessive gene influencing the tubular handling of the four amino acids and that the heterozygous individuals were normal. Standard genetic analyses of these families were in accord with this hypothesis.

In the second type of family, the distribution of cystine excretion varied over a wide range. Values intermediate between the high excretion values, characteristic of the stone formers, and normal values were common. It was suggested that the simplest hypothesis was that there was a single gene segregating in these families that determines cystinuria. The extreme cystinurics (those with high cystine outputs and stone formation) would be homozygous. Persons with intermediate levels of cystine excretion would be heterozygous. This group has been termed semicystinuric. Familial configurations were compatible with this hypothesis.

Harris *et al.* (*Ann. Human Genetics* **20,** *57 (1955)*) subsequently re-examined these families, analyzing the urines for cystine, lysine, arginine and ornithine by the methods previously described. The data are in essential agreement with those obtained from cystine analysis alone. Two distinct abnormal phenotypes were distinguished. In phenotype 1, there was a greatly increased excretion of cystine, lysine, arginine and ornithine and stone formation was common. Phenotype 2 was characterized by moderately increased lysine and cystine excretion only.

The families fell into two groups with respect to phenotype 2. In one group of families phenotype 2 is not found and the segregation of phenotype 1 is consistent with the hypothesis that it represents the homozygote for a recessive gene. This type was called "recessive" cystinuria. In the other group of families where phenotype 2 occurred, the simplest interpretation was that this was due to the heterozygote of another rare recessive gene. In all families in which phenotype 2 was found, it was proposed to call this condition incompletely recessive cystinuria rather than semicystinuria.

The union of biochemistry and genetics

throughout these studies has been most fruitful. It has permitted the establishment of the presence of two abnormal genes associated with cystinuria, and has considerably clarified the mechanisms involved in cystinuria.

ETHER ANESTHESIA AND PYRUVATE METABOLISM

It has been noted by W. R. Drucker *et al.* (*Clin. Res. Proc.* **4**, *202* (*1955*)) that ether anesthesia in man causes a marked disturbance in glucose tolerance but has little effect on the tolerance to fructose. This was interpreted as indicating that ether anesthesia interferes with some specific enzymatic process. In investigating this problem further, W. R. Drucker and associates (*Surg. Forum* **7**, *185* (*1956*)) studied the effects of ether anesthesia on pyruvate metabolism since pyruvate is the final product formed in anaerobic glycolysis, and on conversion to acetyl CoA it supplies fuel for the citric acid cycle.

Studies were carried out on five patients hospitalized for rehabilitation. Each of the individuals received two pyruvate tolerance tests. The first one, which was used as a control, was carried out a day or two prior to anesthesia, and the second test was done during the administration of anesthesia. No operative procedure was performed. The subjects were fasted and required to remain in bed during the day of the study, since activity influences the blood pyruvate level. Blood samples were obtained every 30 minutes during the 90 minutes of surgical anesthesia and at the same interval for the next two hours. The anesthesia was started with a mixture of nitrous oxide, oxygen and ether, with thiopental sodium given just before the gases to minimize the excitement which frequently occurs during the induction of ether anesthesia. The anesthesia was maintained with an ether-oxygen mixture through an endotracheal tube. Thirty minutes after the ether anesthesia was begun a blood sample was obtained and the infusion of sodium pyruvate started. Each subject received 0.12 g. of pyruvic acid per kg. of body weight over a period of one hour. The sodium pyruvate solution infused contained 16.5 g. of sodium pyruvate per liter (13.2 g. of pyruvic acid) and had a pH of 7.40 which was attained by adding the proper amount of hydrochloric acid.

There was no change in the pyruvate level of the blood during the first 30 minutes of anesthesia before the pyruvate was infused. But 30 minutes after the initiation of the pyruvate infusion the average level for the control group (not anesthetized) rose 1.33 mg. per 100 ml. while the average increase in the anesthesia periods was 3.13 mg. per 100 ml. over the fasting level. After 60 minutes of infusion the average rise in pyruvate was 3.32 mg. per 100 ml. over the fasting level for the control periods and only 2.64 mg. per 100 ml. for the anesthesia periods. On stopping the infusion there was a prompt drop in the pyruvate level to the fasting values for the controls but in the anesthetized group the levels were 0.55 mg. per 100 ml. above the fasting level for at least two hours after both the infusion and the ether were stopped. All differences between the pyruvate levels in the control and anesthesia studies were statistically significant as shown by the "t" test.

Following the administration of pyruvate there was an increased lactic acid level in the blood. The differences in the lactic acid levels between the control and anesthesia periods were even greater than that noted for pyruvate. The average lactic acid level at 30 minutes after initiation of the infusion of the pyruvate was increased 5.5 mg. per 100 ml. above the fasting value for the controls, and 14.5 mg. per 100 ml. in the anesthesia study. Two hours after stopping both the anesthesia and infusion the average lactic acid level of the anesthetized group was 12.6 mg. per 100 ml. above the fasting

level and, for the control patients, only 5.6 mg. above the level obtained at the start of the infusion.

Pyruvic acid occupies a keystone position in intermediary metabolism standing at a point where the pathways of carbohydrate, fat and protein metabolism come together. In addition pyruvate can be used in the citric acid cycle for the production of energy. While the present study shows definitely that ether anesthesia causes a block in the metabolism of pyruvic acid, the exact position of the defect is not located. Information gained from such a study aids in the understanding of the metabolic alterations which occur following anesthesia, and also may offer some information concerning the mechanism of ether anesthesia itself.

RETROLENTAL FIBROPLASIA

During the last eight years there have been a number of comments concerning retrolental fibroplasia in this journal. The initial epidemiologic studies of V. E. Kinsey and L. Zacharias (*Nutrition Reviews* **7,** *169 (1949)*; *J. Am. Med. Assn.* **139,** *572 (1949)*) reported a positive correlation in time between the increasing incidence of this disease in premature infants and the use of certain vitamin preparations, the increased use of iron, and increased oxygen administration. The possible role of vitamin E deficiency in the development of this syndrome has also been evaluated (*Nutrition Reviews* **8,** *116, 353 (1950)*). Subsequently, (*Ibid.* **9,** *336 (1951)*) the role of vitamin E in newborn infants was discussed further. Among the efforts to elucidate the role of nutritional factors was a review of work in kittens attempting to relate the incidence of retrolental fibroplasia to a high salt intake (*Ibid.* **11,** *103 (1953)*).

By 1953 attention was focused mainly upon the possibility that excess oxygen administration might be an etiological agent in retrolental fibroplasia, and a large scale cooperative study was organized to investigate this possibility, since observations made in single centers have been capricious and difficult to interpret. The results of this study have recently appeared (V. E. Kinsey, A.M.A. *Arch. Ophth.* **56,** *481 (1956)*). Its purposes were two-fold: (1) To determine whether the incidence of retrolental fibroplasia was positively associated with the duration of exposure of the premature infant to oxygen; (2) to evaluate the effect on mortality of lessening the amount of oxygen given after the age of 48 hours. Two groups of infants were to be maintained under as nearly similar conditions as possible, except for the administration of oxygen to one group for approximately 28 days (a routine in premature infant care at the time). The other group would be given as little oxygen as possible. All premature infants who were born in, or brought to, the 18 cooperating hospitals, who weighed 1500 g. or less at birth, and who survived 48 hours were admitted to the study. Assignment to the two groups was made in a Coordination Center by telegraphy in accordance with a pre-arranged plan in order to remove local bias. Routine orders, including measurement of the oxygen concentration three times daily, were established and used by all of the cooperating centers.

The total population of this study amounted to 786 infants accumulated during a 12 month period. Of the 620 surviving infants, 95 per cent were followed for a minimum of 2½ months. There were 53 infants in the routine oxygen group and 533 infants in the curtailed oxygen group. The mortality rates in the routine and curtailed oxygen groups did not differ significantly. The incidence of active stages of retrolental fibroplasia in the routine oxygen group was approximately twice that in the curtailed oxygen group. The incidence of cicatrical grades of the disease in the routine oxygen group was approximately 3½ times that in the curtailed oxygen group. Both of these results were statistically significant at a

probability of less than 0.01. Both active and cicatrical forms were less frequent in single births than in multiple births. The incidence of both forms of retrolental fibroplasia increased rapidly with increased duration of exposure to oxygen. In infants of single births this increase was most rapid after exposures to oxygen of only one or two days. The incidence of cicatrical forms was not particularly dependent upon the concentration of oxygen administered except after exposures approaching 12 days. The incidence of cicatrical forms was inversely related to birth weight and was not apparently influenced by race, sex, type of incubator, or a number of other miscellaneous factors. Of the group of infants who received no added oxygen after entry into the study at age 48 hours, less than 1 per cent developed retrolental fibroplasia. The severity of the retrolental fibroplasia was not dependent upon the duration in, or concentration of, oxygen or other factors studied.

This controlled study showed conclusively that the length of time the premature infant was kept in an oxygen-enriched environment was the important factor in the production of retrolental fibroplasia. Furthermore, limiting the duration of oxygen administration to that deemed necessary to meet frank clinical emergency was shown to be without effect on the survival rate of the premature infant after the age of 48 hours. There is apparently no concentration of oxygen in excess of that in air that is not associated with the risk of developing retrolental fibroplasia. The rate of withdrawal from oxygen does not appear to play a primary role in the pathogenesis of this disease. Premature infants, particularly those from multiple births, should be kept in an oxygen-enriched environment for the absolute minimum time consistent with adequate prevention of anoxia. This large-scale clinical study, including patients in 18 well run premature centers and designed to answer statistically the question concerning the influence of oxygen administration after the age of 48 hours on the development of retrolental fibroplasia, has answered that question in the affirmative. The previously noted relationships (none of which were proved but which excited considerable attention at the time that they were reported) between retrolental fibroplasia and the administration of water-dispersible multiple vitamin preparations, and the routine administration of iron early in the life of premature infants as well as the suspicion that vitamin E might be connected with this disease, can now be forgotten.

These clinical studies have given rise to attempts to reproduce retrolental fibroplasia in animals. Since this disease occurs in premature infants usually weighing under 1500 g. and is more common in infants in multiple births, it would seem likely that it is a disease due to immaturity of the retina. Indeed, this has been shown to be the case. The immaturity of the retina is reflected in its poor vascularity in infants of low birth weights. Several species of animals, notably the rat, mouse and kitten, have incompletely vascularized retinas at birth. The exposure of newborns of these species to high concentrations of oxygen at birth has been carried out principally by three groups of workers (N. Ashton, *Ann. Rev. Med.* **8,** *441 (1957)*; A. Patz, *Pediatrics* **19,** *504 (1957)*; and L. J. Gyllensten and B. E. Hellström, *Am. J. Ophth.* **41,** *619 (1956)*). The general results of all three groups have been similar. Hyperoxia in newborn animals produces obliteration of the retinal vessels but not of other vessels in the body. This obliteration begins at the periphery of the retina and extends toward the optic disc which is more resistant to hyperoxia. On return to an environment not containing added oxygen, a vaso-proliferative phase ensues with retinal angulation and some destruction of retinal cells. This begins nearest the optic disc and extends outward with invasion of the vitreous by new capillaries. In all but two of several hundred animals studied by these three groups of workers there has been no formation of a retrolental membrane.

These changes in animals appear to be

proportional to maturity. This would suggest that some oxygen-sensitive phase of retinal metabolism was involved. Since high oxygen concentrations failed to affect vessels elsewhere in the body, this would appear to be a rather specific developmental change. The duration of exposure to oxygen was found to be relatively unimportant since Ashton was able to find an effect on the retinal vessels under visual observation within five minutes after a kitten was placed in an environment containing 70 to 80 per cent oxygen. The vaso-obliterative changes that ensued following the initial vasal constriction in oxygen were demonstrated anatomically as irreversible between 24 and 36 hours after the animal was placed in the oxygen-enriched environment. Proportionality between these vascular changes and oxygen concentration was found only within a limited range.

Concentrations of oxygen below 40 per cent produced very little change in the experimental animals and almost all of them showed changes in concentrations over 50 per cent. Between these two concentrations there was a relationship between incidence and oxygen concentration. It has also been observed that where the retina was detached from the underlying choroid, which rarely happens spontaneously in animals, the vaso-obliterative changes did not occur. The development of the retinal vessels was abnormal with extension into the vitreous resembling that seen in human retrolental fibroplasia. This has led to the conclusion that the normal development of retinal vessels depends upon the proximity of the developing retina to the choroid. Ligation of the retinal artery was not followed by reduction in the oxygen tension in the vitreous humor of animals in an oxygen-enriched environment. Sympathectomy or adrenergic blocking agents had no effect. Neither did the carbon dioxide concentration. The mode or rate of transfer from oxygen-enriched environments to air may have slightly retarded the secondary vaso-proliferative change. This could again be temporarily surpressed by increasing the oxygen in the environment, but these changes tended to occur even in animals which remained for relatively long periods of time in a high oxygen concentration. Hellström (*Acta Paediat.* **45**, *43* (*1956*)) found that this process was depressed in starvation and that when the animals were re-fed on removal from high oxygen concentration, the proliferative changes were accentuated.

This growing literature on experimental retrolental fibroplasia shows that high oxygen concentration will produce obliterative changes in the immature retina in experimental animals. Upon transfer to an environment of air relative hypoxia produces proliferative changes in the vessels. These changes cannot be produced by hypoxia alone. Many similarities exist between these findings and human retrolental fibroplasia. The differences are a rarity of retinal detachment and cicatrization in the animal experiment. It has been pointed out by Ashton (*loc. cit.*) that there are fibroblastic elements present in the vitreous extension in the human eye which are lacking in the animal experiments. These fibroblastic elements require nutrients and constrict during healing, pulling the retina away from the cornea. There are certainly factors other than oxygen to explain these differences. At present, studies of the metabolism of the immature retina are projected or are being carried out. Of particular interest is the fact that anaerobic glycolysis occurs at a rate in the retina that is higher than in any other tissue in the body, and is equalled only by that found in tumors.

ANEMIA RESPONDING ONLY TO CRUDE LIVER EXTRACT

Decreased blood hemoglobin levels have been known to be caused by numerous types of nutritional deficiencies. Earlier reviews have discussed anemia as related to specific

nutritional deficits (*Nutrition Reviews* **12,** *14, 257 (1954)*; **14,** *220, 108 (1956)*). Recently D. L. Horrigan, R. M. Whittington, R. Weisman, Jr., and J. W. Harris (*Am. J. Med*, **22,** *99 (1957)*) have reported a type of anemia which has not been previously described. This type of anemia was found in two male patients 38 and 40 years old. The hematologic findings common to these individuals were several. The red cells showed variation in size, shape and color saturation. The mean corpuscular hemoglobin content was consistently low, and the mean corpuscular volumes were predominantly microcytic. The bone marrow showed erythroblastic maturation arrest, but the cytology was not that noted in pernicious anemia. Serum iron levels were elevated with increased saturation of the serum iron binding capacity.

The first patient had no signs of a deficiency of the B vitamin complex and showed a hypochromic anemia with a hemoglobin content of 6 g. per 100 ml. No evidence of any blood loss was noted in this patient or the subsequent one. He was given 30 micrograms of vitamin B_{12} for ten days, and 30 mg. of folacin per day for two additional weeks. Neither of these substances produced a response in reticulocytes. Upon giving 5 ml. per day of crude liver extract intramuscularly a reticulocyte response was noted on the eighth day, reaching a maximum by the nineteenth day with a peak value of 19 per cent. After one month of this therapy the intramuscular injections were reduced to twice weekly. Two months after initiating the intramuscular injections of the crude liver extract, the hemoglobin content of the blood reached 13 g. per 100 ml.

The patient was discharged from the hospital in June but returned in December of the same year with a hypochromic microcytic anemia of the same type as he had on the first admission. The etiology of the anemia could not be determined. Radiochromatic autosurvival time of the patient's erythrocytes was found to be shortened. The half-life of the tagged cells was 28 days, and all the tag was removed in 89 days. When he was again given crude liver extract intramuscularly he showed no reticulocyte response, nor did Leucovorin, intravenously administered iron, or 1000 micrograms of vitamin B_{12} per day induce any increased number of reticulocytes in the peripheral blood. At this time it was believed that the crude liver extract contained a heretofore unidentified factor or factors present in the first batch of liver extract used but not present in the second. Accordingly, the patient was then given a U.S.P. liquid extract of liver. On administering 30 ml. of this preparation three times a day a prompt rise in reticulocytes occurred with a peak of 25 per cent being reached on the ninth day. During this time the hematocrit rose from about 22 per cent to 31 per cent and kept increasing until a value of 42 per cent was reached several weeks later. The mean corpuscular hemoglobin concentration rose from 25 to 30 per cent.

A second patient had a similar history of moderately severe anemia and failed to respond to oral iron, folacin, intramuscular crude liver extract and vitamin B_{12}. At one time during his hospitalization the hemoglobin content fell to 4 g. per 100 ml. which was corrected by a blood transfusion. During the five year period he was studied, his hemoglobin remained between 6 and 9 g. per 100 ml. This man had progressively deteriorating liver function during the period of observation and liver biopsy showed evidence of hemochromatosis. Also a histamine-fast achlorhydria was found but the bone marrow did not show the characteristic signs of untreated pernicious anemia. Upon giving him 30 ml. of the U.S.P. liquid liver extract orally three times daily, a reticulocyte response occurred and the hematocrit rose from 28 to 43 per cent in two months.

These two patients show a unique type of nutritional anemia responding to an unknown factor or factors present in crude liver extract prepared for oral administration. These principles have not been recog-

nized as being essential for red cell synthesis in the human. The active substance is not pyridoxine, a deficiency of which has been known to cause anemia in animals and in man. As to the etiology of the anemia in these two patients, the following factors allow these investigators to speculate that it is due to a failure of iron utilization in the synthesis of hemoglobin: 1) hypochromia of the erythrocytes; 2) erythroid hyperplasia with a decreased number of mature erythroblasts in the bone marrow; 3) elevated serum iron levels; 4) increased saturation of the serum iron binding capacity; 5) hemosiderin deposition in the tissues.

These two patients permitted Horrigan and associates to study rather intensively a new factor which is needed in the synthesis of hemoglobin. Such an experimental scheme could not have been duplicated in the laboratory at present, but probably after the finding of the exact nature or the missing factor such nutritional deficiencies might be produced experimentally.

HYDROCORTISONE CONTENT OF HUMAN ADRENAL VEIN BLOOD

Changes in adrenal cortical activity have been postulated as an important response mechanism in animals undergoing various stressful conditions. D. M. Hume and D. H. Nelson (*Surg. Forum* **5**, *568* (*1954*)), by cannulating the adrenal vein of dogs, have been able to measure the hydrocortisone produced following a contralateral adrenalectomy. Recently J. D. Hardy and M. D. Turner (*Surg. Forum* **7**, *139* (*1956*)) have reported the hydrocortisone content of human adrenal vein from samples obtained at operation. During an operation, a number 17 needle was inserted into the vein as it leaves the left adrenal and the vessel beyond the needle was temporarily occluded. Thus, the blood flowing from the left adrenal vein could be collected over a time interval. Determination of both free and conjugated hydrocortisone was accomplished in both the systemic and adrenal blood. The adrenal vein blood was obtained only at the time of operation. Hydrocortisone was determined by the procedure described by Nelson and L. T. Samuels (*J. Clin. Endocrinol. Metab.* **12**, *519* (*1952*)) as modified by A. M. Bongiovanni (*Proc. Soc. Exp. Biol. Med.* **87**, *282* (*1954*)).

In eight patients on the day before operation, and immediately preoperatively, the systemic venous blood had an average hydrocortisone level of 5.6 and 7.8 micrograms per 100 ml. for the free and conjugated forms, respectively. During the operation the average level of the free and conjugated hydrocortisone in the systemic blood was 24 and 16.7 micrograms per 100 ml. (16 patients). At this time the adrenal vein blood contained 312 micrograms of free hydrocortisone and 90 micrograms of the conjugated form per 100 ml. Four hours postoperatively the systemic venous blood from seven patients contained an average of 9.9 micrograms of the free hydrocortisone and 25 micrograms of the conjugated form. This is the only time when there was a large preponderance of the conjugated form in the circulation. This increased concentration of the conjugated hydrocortisone might have been due to the rapid rate of conjugation of the large amount of hydrocortisone which was secreted by the adrenal during the operation. By 24 to 48 hours after the operation the elevated blood corticoid level had subsided.

By calculating the difference in the systemic venous blood and the adrenal venous blood, it was found that the adrenal was adding about 200 micrograms of free hydrocortisone per 100 ml. of plasma flowing through the gland. The average plasma flow from the gland was 12.5 ml. per minute (25 ml. of blood). Thus the average output of free hydrocortisone by the left adrenal was one-eighth of 200, or 25 micrograms per minute. Using a similar calculation it was estimated that about 13.6 micrograms of conjugated hydrocortisone was being pro-

duced per minute by the left adrenal. While it appears that the adrenal is producing a conjugated form of the steroid these workers believe it to be more probable that a rapid conjugation occurs as the free form enters the circulation. It can be estimated that both adrenals during the operation were adding about 77 micrograms of hydrocortisone per minute to the plasma. The hydrocortisone content of the red cells was not considered though it has been estimated to be 25 per cent of the total blood content.

Since changes of adrenal activity have been implicated in many types o' response reactions, knowledge of the actual steroid production of the gland is important. In this work the hydrocortisone output of the left adrenal gland of a human was obtained under conditions of surgical and anesthetic stress. With future refinement of techniques it might be possible to obtain the minute adrenal output of experimental animals under conditions of minor stress or even in the resting condition.

PANTOTHENIC ACID IN SWINE NUTRITION

Renewed attention has been given by animal nutritionists to pantothenic acid deficiency in swine. This interest is no doubt partly due to findings that practical rations composed chiefly of all-plant materials (corn-soybean meal) may be low in this vitamin for growing and breeding swine (*Nutrition Reviews* **14,** *144* (*1956*)). Several recent studies on pantothenic acid in swine contribute to our knowledge of this subject.

G. L. Sharma *et al.* (*Am. J. Vet. Res.* **13,** *298* (*1952*)) studied the pathology of the intestine of weanling pigs when fed a ration of natural feedstuffs low in pantothenic acid. Pigs on the deficient ration showed poor weight gains, diarrhea, and colitis. In the early stages of the deficiency the large intestine showed degenerative changes such as congestion, edema, and thickening of the wall. Later, small "superficial discrete ulcers" appeared. The columnar epithelium of the intestine showed various pathological changes including necrotic areas and excessive mucus. All symptoms were prevented by pantothenic acid. Previously Wintrobe *et al.* (*Bull. Johns Hopkins Hosp.* **73,** *313* (*1943*)) and others had observed some of these symptoms in pigs fed synthetic rations deficient in pantothenic acid (*Nutrition Reviews* **2,** *125* (*1944*); **3,** *269* (*1945*)).

R. W. Luecke, J. A. Hoefer, and F. Thorp, Jr. (*J. Animal Sci.* **12,** *605* (*1953*)) found that five of ten pigs fed a practical corn-soybean meal basal ration developed marked symptoms of pantothenic acid deficiency. The deficient pigs showed severe locomotor incoordination and slow growth. Chlortetracycline (aureomycin) was added to the basal ration for 1 group because of a reported sparing effect of this antibiotic on pantothenic acid deficiency in the rat (*Nutrition Reviews* **10,** *107* (*1952*); **14,** *124* (*1956*)). Feeding chlortetracycline at a level of 11 mg. per kilogram of ration had no sparing effect on the requirement for pantothenic acid or on the incidence of deficiency symptoms. The authors suggested that this level may have been too low to be effective, since high levels had been used in the rat. The addition of 4.4 mg. of calcium pantothenate per kilogram of ration (making a total of 11.5 mg.) prevented all deficiency signs.

D. V. Catron *et al.* (*J. Animal Sci.* **12,** *51* (*1953*)) could not obtain symptoms of pantothenic acid deficiency in growing swine fed a corn-soybean meal ration under conditions similar to the above experiment. Catron and co-workers used a ration with a higher protein content (18 per cent as compared with 15.4 per cent), which may account for the differences in results.

S. C. Stothers *et al.* (*J. Nutrition* **57,** *47* (*1955*)) studied the pantothenic acid requirement of baby pigs three to four days old given a synthetic milk diet. Various groups of a total of 62 pigs were fed five different levels of pantothenic acid (from 0 to 15 mg. per kilogram of solids). It was

found that the pantothenic acid requirement appeared to be about 12.5 mg. of calcium pantothenate per kilogram of solids. The baby pigs receiving the milk devoid of pantothenic acid developed a severe diarrhea, usually within two to four weeks. This was followed by locomotor incoordination and death, unless the vitamin was given. Pathological studies showed lesions present in the large intestine and in the nervous system similar to those previously described by others. Also the glomerular layer of the adrenal glands was usually thinner in the deficient pigs.

The effect of chlortetracycline on the pantothenic acid requirement of young pigs fed a practical ration has been studied recently by J. I. McKigney, H. D. Wallace, and T. J. Cunha (*J. Animal Sci.* **16,** *35 (1957)*). A total of 54 five-week-old Duroc pigs was used in these studies. The basal ration consisted of 82 per cent of yellow corn, 16 per cent of soybean oil meal, and supplements of minerals and all necessary vitamins except pantothenic acid. The ration was purposely low in protein (about 14 per cent) and was found to contain, by analysis, approximately 6.7 mg. of pantothenic acid per kilogram.

Pigs fed the basal ration grew at the same rate (an average of 1.25 pounds per day in two 12-week experiments) as those receiving extra pantothenic acid. Although no weight differences were seen, most of the pigs on the basal diet developed deficiency symptoms including diarrhea and inflammation of the large intestine. Several of the animals had a typical lameness ("goose-stepping", which is known to be a sign of pantothenic acid deficiency) and blood was observed in the feces of five of 18 pigs on the basal ration. Pigs receiving chlortetracycline for 12 weeks, at a level of 22 mg. per kilogram of diet, averaged 1.37 pounds of gain per day. All symptoms of deficiency were prevented by the antibiotic as well as by pantothenic acid, demonstrating a sparing effect of the antibiotic. Unfortunately, no data were given showing whether or not the effects of the antibiotic and pantothenic acid might have been additive as far as growth is concerned.

Analyses of the pantothenic acid content of the brain, heart, kidney, and liver of pigs on the various groups were made by microbiological assay. No differences were observed except in the liver where the pantothenic acid content was highest in the group receiving chlortetracycline.

C. E. Barnhart, D. V. Catron, G. C. Ashton, and L. Y. Quinn (*J. Animal Sci.* **16,** *396 (1957)*) made a study attempting to show the relationship between different dietary levels of pantothenic acid and rate of gain, certain blood constituents, and urinary excretion in pigs. A purified basal diet was used containing starch, glucose, isolated soybean protein, cellulose, corn oil, minerals and vitamins including 110 mg. of ascorbic acid and 3.6 mg. of pantothenic acid per kilogram of diet. Six different levels of pantothenic acid (4.4 to 15.4 mg. per kilogram of diet) were used, but it is not clear from the authors' report whether or not the various levels of pantothenic acid included the 3.6 mg. of pantothenic acid present in the basal ration. The reason for using a basal ration to which pantothenic acid has been added is not explained by the authors.

It was found that there were no significant differences in the rate of gain, in amount of feed consumed, or in blood characteristics with the six different levels of pantothenic acid tested. Differences were observed in urinary excretion of pantothenic acid with the levels excreted closely related to the dietary levels of pantothenic acid, as would be expected. The feeding of 11 mg. of chlortetracycline per kilogram of diet had no effect on any of these results.

It is unfortunate that Barnhart and coworkers did not give a group of pigs the basal ration containing no pantothenic acid. It would seem that such a negative control group would be necessary as a check, at

least, of possible pantothenic acid contamination or activity of the ration. For instance, perhaps the ascorbic acid in the ration spared pantothenic acid, since it is known that larger amounts have such an effect in the rat (F. Daft, *Fed. Proc.* **10,** *380 (1951)*). It would appear, too, that much effort was wasted in studying six different levels of pantothenic acid (with and without an antibiotic) when the diet already supplied an adequate amount of pantothenic acid activity.

The pig is becoming increasingly important as an experimental animal in basic biochemical and medical experiments. Although differences still exist in results of studies in various laboratories on pantothenic acid in swine, it is obvious that this vitamin plays an important role in swine nutrition and physiology just as it does in other animals. This role appears to have practical significance since diets composed of practical ingredients may be demonstrated to be low in pantothenic acid, especially when the protein content is low. Further work will be necessary before this observation can be extended to other species, including man. At present, there is no evidence that vegetable rations in the human dietary are low in pantothenic acid.

ANTI-CHOLINE COMPOUNDS

Choline can be synthesized by mammalian tissues (*Nutrition Reviews* **9,** *231 (1951)*; **10,** *27 (1952)*; **14,** *278 (1956)*). Despite the synthetic ability of animal tissues, the severe consequences of a lack of dietary choline are well recognized, particularly in such species as the chick, the turkey poult, the rat, the guinea pig, and the rabbit. Present knowledge indicates that the dietary lack of choline is more critical in the young, rapidly growing animals than in older animals. This presumably indicates that the choline requirement for tissue deposition is in excess of the capacity to synthesize this nutrient.

Young rats on a choline-deficient diet develop severely hemorrhagic kidneys, usually in seven to ten days, and this condition is usually fatal.

D. J. Mulford and C. E. Outland (*J. Nutrition* **61,** *381 (1957)*) have tested the effect in rats on kidney hemorrhage of 2-amino-2-methyl-1-propanol, a compound which has been reported to possess anti-choline-like properties for the rat (I. C. Wells, *J. Biol. Chem.* **217,** *631 (1955)*).

Male rats, 3 to 20 weeks of age, served as experimental animals. The basal low-choline diet used had the following percentage composition: casein, 18; dry brewers' yeast, 6; agar, 2; salt mixture, 4; cane sugar, 48.7; lard, 19.9; vitamin A and D oil, 0.1; calcium carbonate, 1.0; and L-cystine, 0.3.

Earlier observations were confirmed in that the age of the animal was important in the development of kidney hemorrhages on the low-choline diet. Ten milligrams of the drug (2-amino-2-methyl-1-propanol) per gram of diet produced severe kidney lesions in three to four-week old animals in six to seven days. This level of the drug produced only slight to moderate lesions in nine to 20-week old rats in 11 to 13 days. Twenty milligrams of the drug per gram of diet were required for the severe condition to appear in the older animals.

The hemorrhage-inducing effects of the drug could be overcome by choline supplementation of the diet. Five milligrams of choline chloride per gram of diet completely prevented kidney hemorrhage in five to 20-week-old rats when the diet contained either 10 or 20 mg. of the drug per gram of diet.

These results essentially confirm the observations of Wells (*loc. cit.*) who found that 2-amino-2-methyl-1-propanol markedly

increased the severity of choline deficiency in young rats.

It is of interest that both of these laboratories have observed that betaine, methionine, and casein had little effect in overcoming the anti-choline action of the drug. (D. J. Mulford, *Proc. Soc. Exp. Biol. Med.* **89,** *471* (*1955*); I. C. Wells, *Fed. Proc.* **14,** *303* (*1955*)).

Based on these observations both Mulford and Wells have come to the tentative conclusion that 2-amino-2-methyl-1-propanol is an inhibitor of choline synthesis in the rat, and as a result precursors, such as betaine or the methyl group of methionine, could not serve as dietary replacements for choline in the presence of the drug. Preliminary results by Wells further suggest that α,α-dimethyltriethyl choline may have a similar anti-choline action.

The careful studies of Mulford and Outland clearly establish that the severe hemorrhagic kidney condition characteristic of choline deficiency could be induced in older rats, namely those considerably beyond the age of three to four weeks. Unfortunately, it is implied that older animals do not suffer this abnormality except in the presence of dietary 2-amino-2-methyl-1-propanol. Abrupt removal of dietary choline in rats five to nine weeks of age has been noted to result in severe kidney lesions and fatal kidney hemorrhage in high incidence without the use of an anti-choline compound in the diet. (R. W. Engel, *Proc. Soc. Exp. Biol. Med.* **50,** *193* (*1942*)).

CALORIE-PROTEIN RELATIONSHIPS

A study of the relationship between available energy content of the diet and food consumption, nitrogen retention, and carcass composition of the rat has been reported by I. R. Sibbald, J. P. Bowland, A. R. Robblee and R. T. Berg (*J. Nutrition* **61,** *71* (*1957*)).

Sixteen groups of young rats were used in their experiment. These were divided into four series which were fed on diets containing 10, 20, 30, or 40 per cent of a non-nutritive cellulose. Thus, the diets of each series contained a different level of digestible energy. Within each series were four groups of four rats each, which received 10, 15, 20 or 25 per cent of protein in their diets. The basal diet contained corn oil (5 per cent), mineral mixture (4 per cent), and vitamin mixture (1 per cent). The nitrogen source consisted of a mixture of casein and lactalbumin supplemented with methionine, histidine, and threonine to provide essential amino acids in the proportions in which they are required by the rat (Sibbald, Berg, and Bowland, *J. Nutrition* **59,** *385* (*1956*)). Sucrose was used as the source of carbohydrate and adjustments in the levels of protein and cellulose were made at the expense of sucrose.

The groups were allowed seven days to adapt to the diets, following which each rat was placed in an individual metabolism cage for seven days. The rations and the feces were analyzed for gross energy content using a bomb colorimeter. Urine and feces of each rat were analyzed for nitrogen. The food consumption and apparent digestible energy (ADE) consumption of each rat were measured, the latter by subtracting total fecal energy from the gross energy intake. Apparent digestible nitrogen (ADN) was similarly calculated.

In order to remove variation associated with differences in body weight, the results were expressed as units per 100 g. of body weight. The weights used in the calculations were the averages of the initial and final weights. The gross carcass composition of each rat was determined at the conclusion of the experiment, but no significant differences were observed.

Food consumption per 100 g. of body weight increased with each increase in the percentage of non-nutritive cellulose in the diet, and was not influenced by the level of dietary protein. The values for energy consumption, on the other hand, were quite constant, ranging from 312 to 350 Calories per 100 g. of body weight. There was some tendency for the values to increase with increasing protein level, which suggests that a correction for urinary energy loss, and that the use of metabolic body size rather than weight, would have reduced the variation (D. M. Hegsted and V. K. Haffenreffer, *J. Nutrition* **157,** *141 (1949)*). Nevertheless, the results show quite clearly, in support of previous observations (*Nutrition Reviews* **12,** *313 (1954)*), that calorie intake is a function of body size and that food intake increases as the energy content of the diet decreases.

Partial correlation coefficients were calculated to permit correction for variations due to differences in protein intake. These corrections did not alter the above relationships. The authors emphasize "that there was no relationship between ADN consumption and food or ADE intake." This relationship holds when all values are brought to a uniform basis, as they were in this study. However, when the level of dietary protein is inadequate (although calorie requirement and, therefore, food consumption, may be determined by body size) body size and hence the *total* caloric requirement, are themselves determined by the protein intake.

The results of the nitrogen balance study showed that nitrogen consumed per 100 g. of body weight increased as the protein content of the diets within each series increased, and that the nitrogen consumption of corresponding groups in the different series increased as the energy content of the diets decreased. Both of these factors led to reduced nitrogen retention. For example, in the series receiving 10 per cent of cellulose, groups fed on diets containing 10, 15, 20 and 25 per cent of protein consumed 1205, 1723, 2256 and 2914 mg. of nitrogen per 100 g. of body weight, respectively, and retained 80, 73, 60 and 47 per cent, respectively, of this nitrogen. When groups receiving a single level of protein, but different levels of cellulose were compared, similar results were obtained. For example, the groups receiving 15 per cent of protein and 10, 20, 30 or 40 per cent of cellulose, consumed 1723, 1968, 2282 and 2413 mg. of nitrogen per 100 g. of body weight, respectively, and retained 73, 67, 59 and 56 per cent, respectively, of this nitrogen.

The authors conclude that "the percentage of ADN retained was not related to the nitrogen intake, but was found to be largely influenced by the ADE of content of the rations." The basis for this statement is the finding that there was a high degree of curvilinear correlation (R = 0.932) between the percentage of ADN retained and the ADE per gram of ADN (ADE/ADN ratio) in the food. It should be noted that this is the kind of correlation which has been termed "spurious correlation," since the two indices which are correlated have a common denominator, ADN, and ADN correlates exactly with itself. An inspection of the data presented will demonstrate that there is a high degree of correlation between the nitrogen intake and the ADE/ADN ratio and also a high degree of correlation between the intake of digestible nitrogen and the nitrogen retention. This statement of the authors is doubtful, and it has certainly not been proven.

The experiment of Sibbald *et al.* was very well designed but their analysis, particularly of the data upon nitrogen retention, leaves much to be desired. Covariance analysis might have demonstrated whether the nitrogen intake or the digestible energy of the rations was most important in determining the nitrogen retention, and would appear to

be the most profitable method of deriving information from the data. Good experimental design is commendable. An appropriate analysis is necessary to gain the full and correct information available from the experiment.

UTILIZATION OF D-VALINE

Recent papers by A. Wretlind (*Acta Physiol. Scandinav.* **36,** *120* (*1956*)) and by M. Womack, B. Snyder and W. C. Rose (*J. Biol. Chem.* **224,** *793* (*1957*)) have been concerned with the utilization of D-valine by the rat. The stimulus for these investigations was the report by J. White, W. S. Fones and H. A. Sober (*J. Biol. Chem.* **199,** *505* (*1952*)) that D-valine supported the growth of rats at about half the rate obtained with L-valine, an observation that appeared to be in conflict with earlier observations at Illinois (W. C. Rose, *Physiol. Res.* **18,** *109* (*1938*)).

Wretlind carried out an extensive investigation in which he determined the rate of growth of rats fed on purified diets containing crystalline amino acids, but devoid of intact protein. He designed some twenty amino acid mixtures in which the level of D-valine was varied and in which the natural and racemic forms of the various amino acids as well as the content of the dispensable amino acids were varied.

He observed initially, using a diet that contained the essential amino acids in the racemic form only, that D-valine failed to support growth. However, the weight loss of rats receiving D-valine was significantly less than that of comparable animals receiving no valine. Also, those receiving D-valine survived for a longer time. When the mixture of racemic amino acids in the diet was replaced by a mixture containing the natural forms of only the indispensable amino acids, D-valine at a level of 2.0 per cent in the diet supported a rate of growth of 1 g. per day during an experimental period of nineteen days. These results indicated that the conversion of D-valine to L-valine was influenced by some component of the amino acid mixture.

In subsequent experiments, in which the various dietary components were tested individually for their effect on the utilization of D-valine, it became apparent that the omission of the D-forms of leucine and isoleucine from diets containing D-valine resulted in a growth stimulation. Finally, by using various combinations of the different forms of leucine and isoleucine, Wretlind demonstrated clearly that the utilization of D-valine was inhibited by the presence of D-leucine in the diet.

The apparent conflict in the earlier results was resolved when it was pointed out that the diet used by Rose (*loc. cit.*) in his original study of D-valine utilization contained DL-leucine, whereas that used by White, *et al.* (*loc. cit.*) contained only L-leucine. Further, Rose had used only 1 per cent of D-valine, whereas White, *et al.* had used 2 per cent of D-valine in their study. Wretlind observed that the utilization of D-valine was improved when the level in the diet was increased from 1 to 4 per cent, and that from 10 to 25 per cent of the D-valine could be used by the rat when the diet contained no D-leucine.

The report of Womack, *et al.* (*loc. cit.*) affords adequate confirmation of the observations of Wretlind. They found that D-valine was required at a level above 1 per cent in the diet to support the growth of rats even in the absence of D-leucine, and that D-leucine in some manner prevented the utilization of D-valine. Although D-valine can support growth, the rate of gain with 2 per cent of D-valine was between 1 and 1.5 g. per day, and in Wretlind's experi-

ments this was increased to about 2 g. per day with 4 per cent of D-valine, in contrast to gains of 3.5 to 5.0 g. per day when 1 per cent of L-valine was included in the diets used by Womack *et al.*

These studies on the effect of D-leucine upon the utilization of D-valine have resolved a controversial point regarding the nutritional value of D-valine. Since W. A. Phillips and C. P. Berg (*J. Nutrition* **53,** *481 (1954)*) have reported that the ingestion of large quantities of the D-amino acids appears to reduce the extent to which even the readily inverted D-forms become available to the rat, further investigation of the D-amino acids may provide other examples of specific antagonisms among the unnatural isomers.

LIPOTROPIC FACTORS IN EDEMA PREVENTION

Severe nutritional edema, anemia, and cirrhosis of the liver have been produced in rats when they were fed diets low in choline and protein (R. W. Engel, *J. Nutrition* **36,** *739 (1948)*; H. D. Alexander and R. W. Engel, *Ibid.* **47,** *361 (1952)*). Dietary choline was effective in preventing these abnormalities. These studies at the Alabama Agricultural Experiment Station, have emphasized that what is commonly referred to as protein-deficiency nutritional edema may represent a deficiency of lipotropic factors rather than of protein.

Several reports from the Alabama laboratory emphasized an interrelationship between choline, or methionine, and the B-vitamins, folacin and vitamin B_{12}. With an adequate dietary protein it was demonstrated that these vitamins significantly reduced the dietary choline requirement in rats and chicks (A. E. Schaefer, W. D. Salmon and D. R. Strength, *Proc. Soc. Exp. Biol. Med.* **71,** *193 (1949)*; A. E. Schaefer and J. L. Knowles, *Ibid.* **77,** *655 (1951)*; A. E. Schaefer, W. D. Salmon, D. R. Strength and D. H. Copeland, *J. Nutrition* **40,** *95 (1950)*).

In a recent report the Alabama workers have investigated the preventive effects of a number of lipotropic factors on the nutritional edema produced on low-choline, low-protein diets (H. D. Alexander and H. E. Sauberlich, *J. Nutrition* **61,** *329 (1957)*).

The three low-protein basal diets employed contained: (1) methanol-extracted peanut meal (10 per cent) and methanol-extracted casein (2 per cent); (2) the same protein sources at 7 per cent and 1.5 per cent, respectively; and (3) oxidized casein (7 per cent) fortified with DL-tryptophan. All diets were fortified with 0.1 per cent of L-cystine. All diets contained 4 per cent of salts, 20 per cent of fat, and sucrose to equal 100 per cent. Further supplements of thiamine, riboflavin, pyridoxine, inositol, calcium pantothenate, niacin, biotin, and vitamins K and E were routinely included in the diets.

The diet containing 10 per cent of peanut meal and 2 per cent of casein contained about 7 per cent of protein. This diet produced fatal nutritional edema and anemia, with an average survival time of 12 weeks. Supplementing this diet with choline prevented the edema and anemia completely, and extended survival time to 50 weeks.

A dietary supplement of folacin (2 mg. per kilogram of diet) alone was ineffective, while dietary vitamin B_{12} (30 micrograms per kilogram of diet) completely prevented the nutritional edema and extended survival to 47 weeks. A combination of vitamin B_{12} and folacin was also effective, and extended survival to 70 weeks in one experiment and to 52 weeks in another experiment.

Supplementation of the diet with DL-methionine (6 g. per kilogram of diet) was

also effective in preventing edema and anemia and extended survival to 54 weeks.

All of the effective dietary supplements to the low-protein diet permitted the weanling rats to grow at a moderate rate and to obtain body weights of 200 to 250 g.

When the dietary protein was reduced to about 5 per cent (7 per cent peanut meal and 1.5 per cent casein), four of eight animals receiving a supplement of vitamin B_{12} alone suffered from generalized edema, while on the basal diet alone, ten of 12 animals had edema. Supplements of choline, methionine and a combination of vitamin B_{12} and folacin were again effective in preventing edema and in promoting growth from weaning weights of about 40 g. to body weights of about 200 g. in 50 to 70 weeks.

Betaine or dimethylaminoethanol (0.3 per cent of the diet) were also effective in preventing the nutritional edema and anemia in rats fed the 7 per cent protein (peanut meal-casein) diet.

When the 7 per cent oxidized casein diet was fed, a 0.4 per cent level of supplementary DL-homocystine had no beneficial effect. Complete protection was afforded however with a further supplement of vitamin B_{12} and folacin.

Lowering the fat in the low-protein basal diets did not affect the incidence of edema and anemia until the fat level was reduced to less than 7 per cent. The low-fat diets resulted in a lowered incidence of edema.

When the 7 per cent oxidized casein diet was supplemented with only 0.08 per cent of methionine, and with either choline or vitamin B_{12} and folacin, survival of weanling rats was extended to 36 weeks with no evidence of anemia or edema.

The retention of water and the development of edema in the Alabama studies is considered in the light of the observed relationship between lipotropic dietary substances and the antidiuretic hormone, pitressin (C. W. Lloyd, *Recent Progress in Hormone Research* **7**, *469* (*1952*)). Lloyd has demonstrated that in rats with liver damage produced on low-protein alipotropic diets the administration of small doses of pitressin resulted in marked water retention. This observation is supported by the studies of K. Guggenheim and E. J. Diamant (*J. Nutrition* **57**, *249* (*1955*)) who noted that livers of choline-deficient rats possess a diminished ability to inactivate pitressin.

These interesting observations obviously lead to the reasonable conclusion that what has been extensively referred to as protein-deficient edema may more appropriately represent a deficiency of lipotropic factors, and that proteins are effective in the relief of edema by increasing the intake of lipotropic factors, particularly methionine.

These studies are of considerable interest in view of the resemblance, noted by the Alabama authors, between the pathology induced on their alipotropic low-protein diets and that reported in human cases of kwashiorkor.

CHOLESTEROL BIOSYNTHESIS

Although cholesterol has no known metabolic function per se, its metabolism is of interest from several standpoints. There is considerable evidence that cholesterol deposits in the arterial walls are related to atherosclerosis. Cholesterol may be synthesized *de novo* in the animal body and it appears that the abnormalities in cholesterol metabolism in arterial disease in man are related to changes in rates of endogenous synthesis of the sterol, rather than to dietary intake of preformed cholesterol. Knowledge of the exact pathway of cholesterol biosynthesis could contribute to a rational approach to therapeutic control of the rate of synthesis. In addition to this role in arterial

disease, cholesterol, or a compound which is related, serves as a precursor of bile salts and steroid hormones. Consequently many of the biochemical steps in cholesterol biosynthesis would be significant in the biosynthesis of these important metabolites.

It has been demonstrated repeatedly that all the carbon atoms of cholesterol may be derived from the two carbon atoms of acetate. The utilization of methyl-labeled and carboxyl-labeled acetate in cholesterol synthesis indicated that 15 carbons came from the acetate methyl, and 12 came from the acetate carboxyl (H. N. Little and K. Block, *J. Biol. Chem.* **183,** *33* (*1950*)). In an effort to elucidate intermediates in the conversion of acetate to cholesterol, a variety of C^{14}-labeled simple compounds were incubated with rat liver slices and their incorporation into cholesterol was determined (R. O. Brady and S. Gurin, *Ibid.* **189,** *371* (*1951*); I. Zabin and Block, *Ibid.* **185,** *131* (*1950*)). It was found that the 4-carbon compound, acetoacetate, was incorporated into cholesterol without prior conversion to acetate. Acetoacetate is formed by the condensation of 2 molecules of acetate. Also isovaleric acid, a 5-carbon branched chain compound, was found to be a very efficient cholesterol precursor. The most likely explanation for these findings is that 2 moles of acetate condense to acetoacetate, which is decarboxylated to a 3-carbon compound such as acetone. This 3-carbon compound would then condense with another mole of acetate to form a 5-carbon branched compound such as isoprene. This scheme, in which one of the acetate carboxyl groups is removed, would explain the observation that more cholesterol carbons are derived from the methyl carbon of acetate than from the carboxyl carbon.

The next problem was to elucidate a high molecular weight precursor of cholesterol. The hydrocarbon squalene, which was originally isolated from shark liver, proved a likely intermediate. This 30-carbon compound can be made to resemble cholesterol quite closely by appropriate folding of the chain. When rats were given C^{14}-labeled acetate, radioactive squalene was isolated and the incubation of this labeled squalene with rat liver homogenates led to the formation of labeled cholesterol (R. G. Langdon and Block, *J. Biol. Chem.* **200,** *129*, *135* (*1953*)). It is possible to condense 6 isoprene units, formed from acetate, to form squalene, and the position of the acetate methyl and carboxyl carbons in the nucleus formed by cyclizing the squalene are the same as actually observed when cholesterol is degraded. These observations strongly support this suggested pathway for sterol formation.

The earliest cyclized cholesterol precursor to be identified is lanosterol, a 30-carbon sterol, originally isolated from sheep wool. This compound is formed when acetate is incubated with liver slices, and C^{14}-labeled lanosterol has been shown to be converted to cholesterol by liver slices (R. B. Clayton and Block, *J. Biol. Chem.* **218,** *305*, *319* (*1956*)).

The conversion of lanosterol to cholesterol requires the loss of three methyl groups. At least two intermediates occur in the conversion. The removal of one methyl group from lanosterol produces 4,4 dimethylcholestadienol, which in turn is demethylated to the 27-carbon sterol, zymosterol. Zymosterol differs from cholesterol in the location of double bonds. Zymosterol is readily converted to cholesterol by liver slices (F. Gautschi and Block, *J. Am. Chem. Soc.* **79,** *684* (*1957*); J. D. Johnston and Block, *Ibid.* **79,** *1145* (*1957*)).

The pathway of cholesterol biosynthesis may then be summarized as follows:

$$\text{Acetate} \rightarrow \text{acetoacetate} \xrightarrow{-CO_2} \text{acetone} \xrightarrow{\text{acetate}}$$

$$\text{isoprene} \rightarrow \text{squalene} \rightarrow \text{lanosterol} \xrightarrow{-CH_3}$$

$$\text{4,4 dimethylcholestadienol} \xrightarrow{-2CH_3}$$

$$\text{zymosterol} \rightarrow \text{cholesterol.}$$

SODIUM, POTASSIUM, AND HYPERTENSION

As knowledge of the pathological aspects of sodium and potassium imbalance has progressed it has become evident that biological interrelationships between these two ions are of critical importance. These interrelationships may apply to the development of hypertension and vascular disease and they appear to be of particular importance in relation to the cardiac and renal lesions of potassium deficiency.

Although normally considered to be an extracellular ion, sodium may enter the cell in amounts exceeding the ability of the normal mechanism for its extrusion when large amounts of sodium are present in the extracellular fluid concurrently with a dietary lack of potassium ions (*Nutrition Reviews* **13,** *148* (*1955*)). To obtain more quantitative data on this subject M. H. Rahman, L. E. Frazier, R. H. Hughes and P. R. Cannon (*Arch. Path.* **63,** *154* (*1957*)) have performed experiments to determine whether excessive amounts of sodium chloride could induce lesions typical of potassium deficiency in the presence of low but normally adequate amounts of dietary potassium.

Young male rats weighing approximately 100 g. were fed diets containing 15 per cent fibrin as the protein source; fibrin was used because it is a high quality protein essentially free of sodium and potassium. Potassium intake was low or normal in five groups of rats in which the sodium content was low, normal, high, or highest. The low values for both sodium and potassium chloride were 20 mg. per day, the normal values were 188 mg. of potassium chloride and 46 mg. of sodium chloride, and the highest values of sodium chloride ranged between 500 and 1500 mg. per day.

Animals were fed in groups and killed at intervals over a period of 53 weeks. Blood pressures were taken twice monthly by a photoelectric method, and terminally directly from the abdominal aorta. No elevations in pressure were found. Histological evidence of potassium deficiency was seen in the myocardium at the forty-seventh week in the animals receiving the most salt and the least potassium. At the end of the experiment all rats in this group showed characteristic cardiac lesions of potassium deficiency, but no other groups showed these myocardial changes. Further indication of potassium deficiency was the appearance of kidney lesions similar to those previously described by B. Spargo (*J. Lab. Clin. Med.* **43,** *802* (*1954*)). Typical changes included distention and dilatation of the proximal and distal convoluted tubules, along with some epithelial cell proliferation in the collecting tubules and a tendency to multilayering of these tubule cells. The presence of coarse intracellular granules in the renal tubules near the papillae was revealed in rats killed in the fifty-second week. No arterial changes were seen.

In view of these morphological findings, additional experiments were designed to learn whether actual differences in potassium retention could be demonstrated in two groups of young rats ingesting the low potassium diet containing either 20 mg. or 1000 mg. of sodium chloride daily. Urines and feces, analyzed weekly for a period of ten weeks, showed a total positive balance of about 6 mEq of potassium in the high sodium group, contrasted with a retention of about 10 mEq of potassium in the low sodium group. The high sodium rats gained an average of 131 g., compared to a gain of 153 g. by the low sodium group.

Carcass analyses of control rats killed at the beginning of the experiment, compared with similar analyses done on experimental rats, permitted an interpretation of the differences in weight gains. The high sodium rats gained less water, fat, protein and ash than the low sodium group. The high sodium fed group gained more carcass sodium but

less potassium. In order to learn whether the loss of potassium resulting from the high sodium diet regimen was caused by the greater fluid intake and resultant diuresis, an additional balance experiment was performed. Both groups of animals were maintained on essentially similar high intakes of a diluted solution of fibrin hydrolysate substituting for dietary nitrogen. The average daily intakes were 53 and 55 ml. for the high and low sodium groups, but in spite of this essentially equal liquid intake the high sodium rats were unable to retain as much potassium (0.25 mEq) as the low sodium rats (4.8 mEq).

Characteristic myocardial lesions developed in all animals on the high sodium intake, with absent or minimal lesions occurring in animals fed the low sodium ration. These experiments suggest that large amounts of sodium may prevent small but adequate amounts of potassium from carrying out their intracellular function either by displacement from the cell or by interfering with its entrance into the cell. The failure to demonstrate kidney lesions or hypertension in these experiments contrasts with the experiments in which G. R. Meneely, R. V. Tucker, W. J. Darby and H. S. Auerbach (*J. Exp. Med.* **98,** *71 (1953)*) observed hypertension and renal failure in growing rats eating diets containing very large amounts of sodium chloride. Both groups of experimenters used male albino Sprague-Dawley rats of similar age and weight, and the experiments lasted about the same length of time. The animals ingested similar amounts of sodium chloride with adequate supplements of potassium chloride in both cases. The animals showing no hypertension consumed a 15 per cent fibrin ration containing 42.5 per cent dextrin and 16 per cent corn oil, in contrast to the hypertensive animals which ate a 25 per cent casein ration containing 52 per cent sugar and 20 per cent hydrogenated vegetable fat. Further study is necessary in order to determine the cause of hypertension in these rats. The dietary differences mentioned suggest studying the effects of varying the amount or kind of protein, carbohydrate, or fat in the ration.

In the meantime, the influence of dietary sodium on the development of renal disease and hypertension has been studied by S. Koletsky with the assistance of C. Van Rooy and A. Press (*Arch. Path.* **63,** *405 (1957)*). Three different techniques were used to produce vascular disease states of graded intensity: (1) bilateral renal artery ligation (consisting of ligation of the main right renal artery and ligation of the anterior branch of the left renal artery); (2) unilateral renal artery ligation (the right kidney was intact but the anterior branch of the left renal artery was ligated); (3) resection of the right kidney and a figure-of-eight ligature applied to the left kidney. Ligation of arteries causes infarction of the entire kidney when the main artery is tied, or infarction of about half of the kidney when one branch of the artery is ligated. The figure-of-eight ligature does not produce infarction, but it is responsible for a perinephritis which may be associated with renal ischemia. Three different diet regimens were used for each operated group: (1) the standard diet of Chow and water, (2) the high salt regimen consisting of Chow and 1 per cent sodium chloride for drinking, (3) a salt-free diet with tap water for drinking.

All animals undergoing renal artery ligation and eating the standard diet developed necrotizing vascular disease. The lesions were more intense in the bilaterally ligated group. Vascular changes were more severe in the rats with the high sodium intake. With the salt-free diet and with bilateral renal artery ligation all but one animal showed necrosis, whereas in the unilaterally ligated group the incidence of necrosis was reduced from 100 per cent to 50 per cent. Animals that were treated by unilateral nephrectomy and a figure-of-eight renal ligature showed 38

per cent incidence of vascular lesions on the standard diet. This value rose to 100 per cent on the sodium supplemented regimen and became zero on the sodium-free diet. The vascular lesions were of the polyarteritis type and involved both arteries and arterioles. In addition focal myocardial necrosis distinct from that of potassium deficiency was seen occasionally, the incidence varying with the level of dietary sodium. With the standard diet only half of the animals with the figure-of-eight renal ligature showed a trend toward elevated blood pressure; all rats with the increased sodium intake, and no rats with the sodium-free diet, were hypertensive. The group with unilateral ligation of a renal artery branch showed a slight elevation of pressure in each animal on the standard diet, and this was increased for the group ingesting extra sodium. The low sodium group, however, showed normal pressure. Finally, rats undergoing bilateral ligation of arteries supplying approximately one and one half kidneys evidenced hypertensive changes in nine of the 16 rats on the sodium-free diet. All rats on the standard diet or high sodium diet showed a gradual elevation of pressure to levels of 160 to 170 mm. Hg. Although blood pressure elevation and vascular changes seemed to occur together after renal injury, there was no absolute correlation between them; each condition occasionally occurred independently of the other.

The reason for the production of vascular disease by this technique is obscure. It might seem that renal injury would result in failure of excretion of some toxic metabolite which injured arteries, but there was no uremia in any of the experimental groups. The possibility that the injured kidney causes retention of a toxic substance responsible for the vascular changes becomes unlikely in view of the experiments of W. J. Kolff and E. R. Fisher (*Lab. Invest.* **1**, *351 (1952)*) who found that necrotizing vascular disease can occur rapidly after total nephrectomy. Studies of electrolyte concentrations of various tissues in animals with hypertension and necrotizing vascular disease revealed that there was a general tendency for sodium to increase grossly regardless of the technique used for the induction of the disease. Thus no matter what the role of sodium chloride is biochemically, the fact remains that ingestion of extra sodium consistently aggravates an already existing electrolyte imbalance which may result from renal injury or from potassium deficiency.

Koletsky's experiments are valuable because they describe a quantitative technique for the elucidation of the electrolyte and renal factors responsible for vascular disease and hypertension. Combined with approaches outlined by Rahman *et al.* and Meneely *et al.*, much may be learned from these which will clarify the role of sodium and potassium interaction in human disease. Other vectors, as yet unknown, may then be studied quantitatively in relation to these important factors.

NUTRITIVE VALUE OF THE PANDANUS

In many areas of the world, especially those which are still remote from industrial civilization, large portions of the population may, by habit or necessity, exist on diets consisting largely of one or two ingredients. Investigations of such communities are of interest in that they offer the opportunity, rare in human studies, of examining a population for which the diet is fairly uniform and can be readily analyzed. In some cases such diets are deficient in one or more essential nutrients and, thus afford the chance to study the resultant deficiency state and to attempt rational measures for its prevention. An illustration of this point is the dependence of the South African Bantu on the cassava, and the

resultant high incidence of protein malnutrition in that area (*Nutrition Reviews* **10,** *261 (1952)*).

In the islands of Micronesia, large sections of the population use the pandanus fruit as a diet staple, in some cases amounting to 50 per cent of the total calories. A study of the nutritional state of the inhabitants of these islands, therefore, must include an evaluation of the nutritive characteristics of this fruit.

Such an evaluation has been made recently by C. D. Miller, M. Murai and F. Pen (*Pacific Science* **10,** *3 (1956)*). The edible varieties of the pandanus fruit weigh up to 30 pounds and resemble, to some extent, large pineapples. Each is composed of up to 50 loose sections, or keys, which contain the edible portions. Half of each key is edible, the remainder being fibrous matter and seeds. The sweet edible parts are eaten raw as a fruit, cooked to a consistency of mashed sweet potato, made into a paste for preservation, or dried and milled into a flour.

All these preparations were analyzed by the authors for moisture, crude fiber, total ash, fat, protein, carbohydrate, calcium, phosphorus, iron, and several vitamins. Although some variation was found in the samples other than the expected differences from different water content, the values taken from the raw fruit are probably representative. Twenty keys (not an uncommon daily intake) were found to supply (for 1500 g.) approximately 1060 calories, 5.6 g. protein, 4.0 g. fat, 144 mg. calcium, 388 mg. phosphorus, and 14 mg. iron.

These values are comparable to those of fresh temperate climate fruits such as apples, pears, and peaches. In the same amount of fresh fruit were found the following amounts of the vitamins: 18.64 mg. carotene, 0.4 mg. thiamine, 0.6 mg. riboflavin, 14 mg. niacin, and 40 mg. ascorbic acid. The fruit is thus a good source of provitamin A, ascorbic acid, and niacin. The pandanus paste contained about the same relative amounts of the nutrients but larger total amounts. An extremely large amount of calcium in the flour was ascribed by the authors to the grinding processes, which might contribute some coral sand. These latter two preparations are valuable as emergency high-calorie rations for long voyages and are comparable to dates in this regard.

It is evident that the pandanus fruit is a poor source of protein, fat, and certain vitamins of the B-complex. However, the authors state that in isolated islands, in which the pandanus might supply half or more of the caloric needs, the other staples are the coconut, which supplies some fat, and fish, which provide protein and additional minerals and vitamins.

NOTES

Letter to the Editor

Sir:

In the March issue of Nutrition Reviews (vol. 15, p. 77) the paper by Kleiber, Smith and Chernikoff, *Amer. J. Physiol.* **186,** *9 (1956)* is reviewed. The review contains the following statement: "The work is a valuable addition to the sum of information on the metabolic rate of rats but the teleological discussion thinly disguised as physiology is unhelpful."

I do not claim that the criticized discussion is helpful—that depends on the reader; but I claim, 1) that it is not disguised, and 2) that it is not teleological. The discussion is based on natural selection which is the alternate to teleology. This has been clearly stated by Hans Reichenbach in his book, "The Rise of Scientific Philosophy" (University of California Publication 1951 p. 201) "the need for teleology is eliminated by Darwin's principle."

Teleology has recently been revived by

very prominent scientists such as A. V. Hill (*Science* **124,** *1233 (1956)*) and H. Krebs (*Bull. Johns Hopkins* **95** *(1954)*). A critique of the latter's argument is found in *Annual Rev. Physiol.* **18,** *35 (1956)*.

Yours respectfully,

MAX KLEIBER, D.Sc.
Professor of Animal Husbandry
University of California
Davis, California

Letter to the Editor

Sir:

About 1917 I began giving sea water to rats to be sure they obtained all the required minor elements. Later, owing to difficulty of transportation I boiled it down, but noticed that before all water was evaporated, magnesium acted as a weak base and precipitated as MgO, and an equivalent of HCI volatilized carrying with it HI. Also the fluorine formed some fluorapatite-like compound (although sea water is poor in phosphate) that would not dissolve when water was added. After serving in Colonel Murlin's Division in the Army I studied the relation of fluorapatite to the teeth. Dr. E. F. Robb and I showed that fluorapatite did not begin to dissolve in saliva until the pH was lowered to 5. Dr. Nelson Taylor, by X-Ray spectrograms, showed that the mineral of the teeth had an apatite crystal lattice. I began feeding powdered fluorapatite and phosphate rock to rats and studying their teeth by chemical analysis, and bones and teeth by X-Ray. I consider phosphate rock to be metamorphosed fossil bone.

In helping some anthropologists dig into an Indian mound on the St. Croix River, I noticed an oak tree growing on top and sending its roots down 20 feet to an Indian skeleton. An oak rootlet penetrated each root canal of the otherwise excellent Indian teeth and extracting the minerals, transported them to the oak leaves (which contain about 500 parts per million of fluorine) and acorns. Indians (who had less dental caries than we have) ate acorns of 18 species of oaks, but I saw only a squirrel eating the acorns and flashing his excellent teeth at me. But some Indian plant extracts high in fluorine (Chapparal tea, teamster's tea, Mexican tea, New Jersey tea, and Labrador tea) were adopted by some settlers.

These studies were hampered by the lack of accurate micro-methods for determining fluorine and I obtained for W. D. Armstrong an all-platinum still for distilling SiF_4. Armstrong determined the fluorine in so small a sample as a rat's tooth and showed that there was more fluorine in sound human teeth than in carious teeth.

DR. J. F. MCCLENDON
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Recent Nutrition Books

Biochemical Individuality: The Basis for the Genetotrophic Concept. By Roger J. Williams, Professor of Chemistry, Director Biochemical Institute, University of Texas. John Wiley & Sons, Inc., New York, 1956. Pp. 209.

Cornell University Symposium Papers on Biochemistry and Nutrition, 1955. Published by the New York State College of Agriculture, a unit of the State University of New York at Cornell University, Ithaca, New York. December 1956. Pp. 100.

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† Deceased

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